

The University of Chicago

CALCIUM UTILIZATION
BY NORMAL WOMEN ON THREE
LEVELS OF INTAKE

A DISSERTATION SUBMITTED TO THE
FACULTY OF THE DIVISION OF THE BIO-
LOGICAL SCIENCES IN CANDIDACY FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF HOME ECONOMICS AND
HOUSEHOLD ADMINISTRATION

1935

By

ALICE ELIZABETH RYDER

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REVIEW OF LITERATURE

A review of the literature on calcium balance studies on human adults reveals much that, in the light of our present knowledge, is difficult to interpret. Inspection of the results of the many studies reported shows great differences in the response of subjects to the various allowances of calcium provided. To cite extremes, definite positive calcium balances for twelve days on an intake as low as 0.26 grams of the mineral daily have been reported by Rose (21), while negative balances almost equal to one another in magnitude have been obtained for sixteen days by Sherman and Winters (24) on a daily calcium allowance of 0.08 grams, and by Hunscher, Donelson, Erickson, and Macy (14) for fifteen days on an average daily intake of 1.1 grams of calcium. Similarly, the amounts of calcium proposed as the minimum requirement for the attainment of physiological equilibrium by adults has varied within the wide limits of 0.28 grams of calcium daily, suggested by Bertrand (4), and 1.48 grams, recommended by Gautier (12), while in this country Sherman's (23) figure of 0.45 grams as the minimum daily calcium requirement for a 70 kilogram man is generally accepted. Moreover, much difference in practice and therefore presumably in opinion, is found to exist in respect to the use of preliminary periods for physiological adjustment to experimental diets and conditions. Bauer, Albright, and Aub (1) used preliminary periods of thirty-six hours before making collections on low calcium intakes; Paget, Langeron, and Cordonnier (19) advocate a preliminary period of three days for calcium balance studies; Rose (21) considered it advisable to exclude from the averages the results of the first four-day period when the subjects had been changed to a lower calcium allowance than that to which they had been accustomed; Manoussakis (18) advised the use of an experimental diet for five days before beginning collections; and Bauer, Marble, and Claflin (3) in an extended calcium balance study used preliminary periods of ten days. Further, presumably because of cost and inconvenience, many of the calcium balance studies made have been of brief duration, and from single short periods, e.g., Bogert et al (5, 6, 7); conclusions, the validity of which may be questioned, have sometimes been drawn, for other workers, as Rose (21), have found comparable differences in consecutive periods of similar length when the subjects have been on a constant diet.

PURPOSE

Because of the questions which arose from a review of the literature on calcium balance studies, the present extended study of the calcium metabolism of normal adults was undertaken, with a fourfold purpose: (1) to study the calcium utilization of the same subjects at widely varying levels of intake; (2) to discover if possible the effect of previous dietary history on calcium utilization, particularly as it might affect the calcium requirement for maintenance of equilibrium; (3) to determine the duration and nature of physiological adjustment to abrupt changes in the level of calcium intake; and (4) to find the magnitude of the variation in calcium balances that might normally be expected in periods of varying length on constant diets, and thereby gain some idea of the length of period which might give a representative sampling of calcium utilization under practically constant conditions.

EXPERIMENTAL WORK

General Plan

The present investigation extended over a period of more than three months during which time the calcium utilization of four adults was studied at three varying levels of intake. In order that the normal variation in excretion on a constant diet might be observed the experiment included, first, one rather long series of 3-day balance periods on a constant moderate calcium intake. Then, so that the physiological adjustment to changes in the level of calcium intake might be studied, the calcium allowance was abruptly changed, first, from the moderate to a high level of intake, and later, from this high level to a low one, and the utilization of the mineral studied during the transition periods. The calcium in the diet of the first series, designated Series A, was planned to approximate the estimated minimum requirements of the subjects. This estimation was based upon the average daily excretion of Subject 2 during a 24-day balance study conducted three months previously, the results of which are appended (Table X). The present study was started within two weeks of the beginning of the fall quarter of the university, and after a preliminary period of six days on the supposedly maintenance diet, collections were made for ten consecutive 3-day periods, omitting in the case of each subject, however, the periods in which catamenia occurred, although this has been shown by several workers to have no apparent effect on calcium excretion. A 5-day recess, which included Thanksgiving

week-end, followed the one day allowed for intestinal lag at the end of the tenth period. During this 5-day interval the subjects approximated the diet that they had been receiving, and on returning to the laboratory were again placed on the same weighed diet for three days before collections for Period 11 of Series A were made. Immediately following this period the diet was changed, so as to provide a high level of calcium intake for Series B, and collections made for five 3-day balances. When the collections for Period 5 of Series B were completed the subjects approximated this diet for two weeks, which included the Christmas vacation, after which a preliminary period of three days on the same diet preceded collections for Period 6 of the same series. Then, at once, the regime of a diet with a low calcium content was instituted, and collections were made for the five 3-day periods which constituted Series C. A calendar of this procedure is given in Table I.

TABLE I
CALENDAR OF EXPERIMENTAL PROCEDURE

Procedure	Days
Series A--Calcium intake estimated to approximate maintenance	
Preliminary period on weighed diet . .	6
Ten three-day collection periods . . .	30
Allowance for intestinal lag	1
Diet approximated	5
Preliminary period on weighed diet . .	3
Collection	3
Series B--High calcium intake	
Five three-day collection periods . .	15
Allowance for lag	1
Diet approximated	14
Preliminary period on weighed diet . .	3
Collection	3
Series C--Low calcium intake	
Five three-day periods	15
Lag	1
Duration of experiment	100
Collections	66

Subjects

The subjects, who have been arranged in order of increasing weights, were four normal, healthy, college women, who volunteered to take part in the experiment, and who were interested, conscientious, and co-operative throughout its course. All had histories of years of high calcium intake and were habitual milk drinkers. Subjects 1, 3, and 4 had been counsellors in children's camps during the preceding summer, spending a great deal of time outdoors in the sunshine, and they had continued to drink considerable quantities of milk until the beginning of the experiment. In definite contrast, Subject 2, in preparation for calcium balance work, had about six months previously begun to take a very low calcium diet, and during the summer had been on a calcium balance experiment, when for forty-five days she had been on diets which caused definite calcium losses. Following this, a high calcium diet had been taken for two weeks in an effort to compensate for these losses, and then, up to the beginning of the present experiment, a calcium intake which was estimated to meet maintenance needs for the mineral, but furnish no excess, had been maintained.

Diets

Except for the purposeful restriction of calcium for part of the time, the diets were planned to meet the nutritional needs and the tastes of the subjects, and were identical for each subject in all the periods of a series. These diets, with the food values as calculated from Sherman (22) and Rose (20), are given in Table II. The average daily caloric value of the diets, which ranged from 2,000 to 2,300 calories, was intentionally kept somewhat low, and cane sugar was used as desired by the subjects but not included in the calculations or analyses. The fact that body weights remained practically constant during the three months of the study indicated the caloric adequacy of the food intake. While most of the dietary essentials remained at approximately the same levels throughout the course of the study, the calcium was varied to fit the plan of the experiment, and the phosphorus, while at all times in excess of theoretical maintenance requirements, varied somewhat in the same direction as the changes in the calcium intake. The subsequent analyses of the food for calcium and phosphorus showed close agreement with the calculations, the average daily intake of these minerals falling within 0.03 grams of the amounts calculated except in Series B where phosphorus was 0.06 grams higher than planned for Subjects 1, 2, and 4 and both phos-

TABLE II
EXPERIMENTAL DIETS

Series A - Calcium Intake Estimated to Approximate Maintenance

Food	Weight	Calo- ries	Pro- tein	Calcium	Phos- phorus	Iron
	gms.		gms.	gms.	gms.	gms.
All subjects-every day						
Orange juice.....	50	22		0.014	0.008	0.00011
Apple sauce.....	100	100	0.30	0.005	0.008	0.00021
Banana.....	150	150	1.97	0.012	0.048	0.00090
Lettuce.....	50	10	0.60	0.022	0.021	0.00035
Shredded wheat.....	30	110	3.50	0.012	0.096	0.00132
Whole wheat bread...	160	416	14.56	0.062	0.250	0.00272
Crackers.....	12	50	1.20	0.002	0.012	0.00018
Beef.....	150	234	31.96	0.018	0.346	0.00378
Egg.....	50	70	6.40	0.032	0.086	0.00144
Butter.....	60	462	0.60	0.009	0.011	0.00015
Mayonnaise.....	15	100	0.20	0.001	0.006	0.00009
Coffee.....	200					
Subjects 1 and 2 daily						
Cream.....	40	78	1.00	0.039	0.035	0.00008
Milk.....	80	55	2.60	0.096	0.074	0.00016
Potatoes.....	150	125	3.30	0.021	0.087	0.00195
Subjects 3 and 4 daily						
Cream.....	50	98	1.30	0.049	0.046	0.00010
Milk.....	100	69	3.30	0.120	0.093	0.00020
Potatoes.....	180	150	4.00	0.025	0.105	0.00234
All subjects						
Tomatoes (1st day)..	150	35	1.80	0.017	0.039	0.00059
Green beans(2nd day)	80	34	1.80	0.037	0.042	0.00088
Onion soup (2nd day)	100	5	0.20	0.003	0.005	0.00006
Cooked rice(2nd day)	30	35	0.80	0.001	0.010	0.00009
Beets (3rd day).....	100	40	1.60	0.029	0.039	0.00060
Tea (occasionally)..	...	...				
Cane sugar.....	...	...				
Totals						
Subjects 1 and 2						
1st day.....	...	2035	72.1	0.362	1.127	0.01402
2nd day.....	...	2079	73.1	0.386	1.145	0.01446
3rd day.....	...	2040	71.9	0.374	1.127	0.01488
Daily average per period.....	...	2053	72.4	0.374	1.133	0.01445
Subjects 3 and 4						
1st day.....	...	2104	73.8	0.400	1.173	0.01447
2nd day.....	...	2138	74.8	0.424	1.191	0.01491
3rd day.....	...	2099	73.6	0.412	1.173	0.01533
Daily average per period.....	...	2114	74.1	0.412	1.179	0.01490

TABLE II (CONTINUED)

Series B - High Calcium Intake						
Food	Weight	Calo- ries	Pro- tein	Calcium	Phos- phorus	Iron
	gms.		gms.	gms.	gms.	gms.
All subjects every day						
Orange juice.....	50	22		0.014	0.008	0.00011
Apple sauce.....	100	42	0.30	0.005	0.008	0.00021
Banana.....	50	50	0.66	0.005	0.016	0.00031
Lettuce.....	50	10	0.60	0.022	0.021	0.00035
Shredded wheat.....	30	110	3.50	0.012	0.096	0.00132
Whole wheat bread...	160	416	14.66	0.062	0.250	0.00272
Beef.....	75	117	16.00	0.009	0.173	0.00189
Potato.....	100	83	2.20	0.014	0.058	0.00130
Butter.....	40	308	0.40	0.006	0.007	0.00010
Mayonnaise.....	10	79	0.10	0.001	0.004	0.00006
Milk.....	500	345	16.4	0.600	0.465	0.00120
Cookies.....	25	100	3.0	0.005	0.015	0.00019
All subjects-additional 1st day						
Milk.....	100	69	3.3	0.120	0.093	0.00020
Tomatoes.....	120	28	1.5	0.014	0.031	0.00047
Spinach.....	120	29	2.5	0.080	0.081	0.00422
Crackers.....	12	50	1.2	0.002	0.012	0.00018
Tapioca cream.....	150	283	8.8	0.217	0.213	0.00133
2nd day						
Peas.....	120	61	4.1	0.033	0.152	0.00204
Crackers.....	24	100	2.4	0.004	0.024	0.00036
Cream of onion soup.	100	69	3.1	0.120	0.093	0.00020
Chocolate pudding...	120	174	3.3	0.102	0.097	0.00032
3rd day						
Banana.....	100	100	1.3	0.009	0.031	0.00061
Carrots.....	100	46	1.1	0.056	0.046	0.00060
Graham crackers.....	24	100	2.4	0.006	0.026	0.00036
Cocoa.....	250	106	3.9	0.101	0.101	0.00029
Subject 3-Add'l daily						
Cookies.....	50	200	6.0	0.011	0.030	0.00038
Totals						
Subjects 1, 2, and 4						
1st day.....	...	2141	75.1	1.188	1.551	0.01616
2nd day.....	...	2068	70.7	1.014	1.487	0.01268
3rd day.....	...	2034	66.5	0.927	1.325	0.01162
Daily average per period.....	...	2087	70.8	1.043	1.454	0.01349
Subject 3						
1st day.....	...	2341	81.7	1.199	1.581	0.01654
2nd day.....	...	2268	76.7	1.025	1.517	0.01304
3rd day.....	...	2234	72.5	0.938	1.355	0.01200
Daily average per period.....	...	2287	76.8	1.054	1.484	0.01387

TABLE II (CONTINUED)

Series C - Low Calcium Intake

Food	Weight	Calo- ries	Pro- tein	Calcium	Phos- phorus	Iron
	gms.		gms.	gms.	gms.	gms.
All subjects every day						
Apple sauce.....	100	100	0.3	0.005	0.008	0.00021
Banana.....	100	100	1.3	0.009	0.031	0.00061
Cornmeal mush.....	200	100	2.6	0.005	0.053	0.00025
Whole wheat bread...	160	416	14.6	0.062	0.250	0.00272
Beef.....	150	234	31.9	0.018	0.346	0.00378
Bacon.....	30	130	2.1	0.001	0.023	0.00026
Butter.....	40	308	0.4	0.006	0.007	0.00010
Potatoes.....	150	125	3.3	0.021	0.087	0.00195
Tomatoes.....	120	28	1.5	0.014	0.031	0.00047
Syrup.....	30	85				
Coffee.....	200					
All subjects-Additional 1st day						
Rice pilaf.....	240	250	3.8	0.008	0.059	0.00047
Fruit tapioca.....	240	210	0.4	0.014	0.030	0.00045
2nd day						
Scalloped corn.....	120	165	8.2	0.009	0.125	0.00106
Prune pudding.....	150	195	0.5	0.016	0.030	0.00085
3rd day						
Onion soup - rice...	150	40	1.0	0.004	0.015	0.00015
Crackers.....	12	50	1.2	0.002	0.012	0.00018
Apricot rice dessert	180	220	2.4	0.016	0.043	0.00051
Totals						
1st day.....	...	2086	62.2	0.163	0.925	0.01127
2nd day.....	...	1986	66.7	0.168	0.991	0.01226
3rd day.....	...	1936	62.6	0.163	0.906	0.01119
Daily average per period.....	...	2003	63.8	0.165	0.941	0.01157

phorus and calcium higher for Subject 3.

Analytical Methods

Generally accepted methods were used for the collection and preparation of samples, and the analysis of food, urine, and feces. Calcium was precipitated by the McCrudden (16) method, at a pH of 5, using methyl red as an indicator, as recommended by Shohl (25). The precipitated calcium oxalate was then titrated with N/50 potassium permanganate. Phosphorus was determined by the colorimetric method of Fiske and Subbarrow (11).

RESULTS AND DISCUSSION

The complete results of the calcium and phosphorus determinations are shown in Table III, and summarized in Tables IV and V by grouping the results of consecutive periods which seem to show significant differences, thus separating, in Series B and C, the adjustment periods from those of later periods, in which body processes had apparently become more or less stabilized, and adjusted to the changed intake. The calcium and phosphorus balances are presented graphically in Figures 1 and 2.

The Effect of Varying Intakes on the Calcium Balances of the Same Subjects

Inspection of the data for Series A shows that with all of the subjects, on a constant daily intake of calcium which amounted to 0.351 grams daily for Subjects 1 and 2, and 0.383 grams daily for Subjects 3 and 4, there was loss of the mineral. In the 27 to 30 days for which collections were made in this series, this amounted to average daily losses of 0.057 grams, 0.024 grams, 0.078 grams, and 0.051 grams for the respective subjects.

Following the abrupt change from the moderate calcium allowance of Series A to the constant high level of intake, which averaged 1.058 grams daily for Subjects 1, 2, and 4 and 1.095 grams daily for Subject 3, there was, in Series B, at first, calcium storage by all of the subjects. With Subjects 2 and 3 this retention, averaging 0.145 grams and 0.261 grams daily, was completed in the first 3-day period of the series, while Subjects 1 and 4 continued to store the mineral during the second period as well, retaining altogether an amount which averaged 0.084 grams daily for Subject 1 and 0.139 grams for Subject 4, during the six days. This storage was, however, only temporary and in the succeeding periods Subject 2 remained in equilibrium, while Subjects 1, 3, and 4 reverted to negative balances, which amounted to average daily losses of 0.045 grams, 0.178 grams, and 0.061 grams respectively.

The sudden change from the high calcium intake of Series B to an average daily intake of only 0.191 grams daily in Series C resulted in marked losses of calcium by all subjects in all periods. For Subjects 1, 2, and 4, this loss was definitely higher in the first period than later, the average daily negative balances being 0.153 grams, 0.168 grams, and 0.299 grams, while in the later periods of the series the corresponding losses averaged

TABLE III
SUMMARY OF CALCIUM AND PHOSPHORUS DATA
Daily Averages in Grams

Subject 1 - Weight 61 Kilograms

Series A

Period	Calcium					Phosphorus				
	Intake	Output			Balance	Intake	Output			Balance
		Urine	Feces	Total			Urine	Feces	Total	
1	0.358	0.130	0.248	0.378	-0.020	1.121	0.604	0.408	1.012	+0.109
2	0.331	0.126	0.205	0.331	+0.000	1.067	0.616	0.347	0.963	+0.104
3	0.335					1.095				
4	0.349					1.091				
5	0.351	0.176	0.210	0.386	-0.035	1.121	0.637	0.356	0.993	+0.128
6	0.351	0.179	0.243	0.422	-0.071	1.070	0.782	0.422	1.204	-0.134
7	0.346	0.215	0.210	0.425	-0.079	1.161	0.844	0.349	0.993	+0.168
8	0.361	0.195	0.182	0.377	-0.016	1.122	0.678	0.318	0.996	+0.126
9	0.344	0.216	0.201	0.417	-0.073	1.160	0.710	0.364	1.074	+0.086
10	0.363	0.274	0.221	0.495	-0.132	1.173	0.789	0.379	1.168	+0.006
11	0.369	0.198	0.257	0.455	-0.086	1.149	0.727	0.395	1.122	+0.027
Av.	0.351	0.190	0.220	0.410	-0.057	1.121	0.687	0.371	1.058	+0.069

Series B

1	1.081	0.305	0.697	1.002	+0.079	1.511	0.838	0.575	1.413	+0.098
2	1.053	0.273	0.690	0.963	+0.090	1.486	0.946	0.498	1.443	+0.043
3	1.051	0.277	0.826	1.103	-0.052	1.533	0.824	0.615	1.439	+0.094
4	1.056	0.314	0.822	1.136	-0.080	1.495	0.863	0.604	1.467	+0.028
5	1.042	0.249	0.801	1.050	-0.008	1.537	0.876	0.592	1.468	+0.069
6	1.065	0.235	0.870	1.105	-0.040	1.530	0.919	0.672	1.591	-0.061
Av.	1.058	0.276	0.784	1.060	-0.002	1.515	0.878	0.593	1.470	+0.045

Series C

1	0.194	0.118	0.228	0.346	-0.152	0.913	0.690	0.358	1.048	-0.135
2	0.196	0.123	0.136	0.259	-0.063	0.925	0.769	0.293	1.062	-0.137
3	0.192	0.140	0.125	0.263	-0.073	0.967	0.702	0.281	0.983	-0.016
4	0.187	0.150	0.122	0.272	-0.085	0.964	0.761	0.275	1.036	-0.072
5	0.188	0.147	0.140	0.287	-0.099	0.924	0.682	0.339	1.021	-0.097
Av.	0.191	0.135	0.150	0.285	-0.096	0.939	0.721	0.309	1.030	-0.091

TABLE III (CONTINUED)

Subject 2 - Weight 64 to 65 Kilograms

Series A

Period	Calcium					Phosphorus				
	In-take	Output			Balance	In-take	Output			Balance
		Urine	Feces	Total			Urine	Feces	Total	
1	0.358	0.095	0.235	0.330	+0.028	1.121	0.652	0.366	1.018	+0.103
2	0.331	0.085	0.260	0.345	-0.014	1.067	0.639	0.446	1.085	-0.018
3	0.335	0.104	0.298	0.402	-0.067	1.095	0.629	0.477	1.106	-0.011
4	0.349	0.101	0.254	0.355	-0.006	1.091	0.563	0.401	0.964	+0.127
5	0.351					1.121				
6	0.351	0.110	0.273	0.383	-0.032	1.070	0.741	0.444	1.185	-0.115
7	0.346	0.103	0.234	0.337	+0.009	1.161	0.782	0.366	1.148	+0.013
8	0.361	0.120	0.282	0.402	-0.041	1.122	0.690	0.483	1.173	-0.051
9	0.344	0.116	0.268	0.384	-0.040	1.160	0.715	0.438	1.153	+0.007
10	0.363	0.133	0.233	0.366	-0.003	1.173	0.692	0.312	1.004	+0.169
11	0.369	0.117	0.327	0.444	-0.075	1.149	0.799	0.459	1.258	-0.109
Av.	0.351	0.108	0.267	0.375	-0.024	1.121	0.690	0.419	1.109	+0.012

Series B

1	1.081	0.165	0.770	0.935	+0.146	1.511	0.806	0.598	1.404	+0.107
2	1.053	0.170	0.885	1.055	-0.002	1.486	0.776	0.670	1.446	+0.040
3	1.051	0.157	0.905	1.063	-0.012	1.533	0.756	0.703	1.459	+0.074
4	1.056	0.160	0.955	1.114	-0.058	1.495	0.778	0.695	1.473	+0.022
5	1.042	0.167	0.818	0.985	+0.057	1.537	0.773	0.650	1.423	+0.114
6	1.065	0.148	0.952	1.100	-0.035	1.530	0.776	0.723	1.499	+0.031
Av.	1.058	0.161	0.881	1.042	+0.016	1.515	0.777	0.673	1.450	+0.065

Series C

1	0.194	0.079	0.283	0.362	-0.168	0.913	0.638	0.439	1.077	-0.164
2	0.196	0.075	0.173	0.248	-0.052	0.925	0.579	0.347	0.926	-0.001
3	0.192	0.092	0.208	0.299	-0.107	0.927	0.636	0.421	1.057	-0.130
4	0.187	0.084	0.172	0.256	-0.069	0.964	0.685	0.361	1.046	-0.082
Av.	0.192	0.082	0.209	0.291	-0.099	0.932	0.635	0.392	1.027	-0.095

TABLE III (CONTINUED)

Subject 3 - Weight 68 to 69 Kilograms

Series A

Period	Calcium					Phosphorus				
	In-take	Output			Balance	In-take	Output			Balance
		Urine	Feces	Total			Urine	Feces	Total	
1	0.394	0.122	0.284	0.406	-0.012	1.140	0.594	0.448	1.042	+0.098
2	0.364	0.124	0.269	0.393	-0.029	1.081	0.677	0.415	1.092	-0.011
3	0.367	0.106	0.324	0.430	-0.063	1.117	0.687	0.498	1.185	-0.068
4	0.385	0.188	0.349	0.537	-0.152	1.112	0.569	0.524	1.093	+0.019
5	0.371					1.157				
6	0.375	0.182	0.281	0.463	-0.088	1.125	0.786	0.449	1.235	-0.110
7	0.385	0.114	0.301	0.415	-0.030	1.198	0.715	0.459	1.174	+0.024
8	0.395	0.166	0.298	0.464	-0.069	1.160	0.688	0.466	1.154	+0.006
9	0.380	0.209	0.297	0.505	-0.125	1.195	0.716	0.448	1.164	+0.031
10	0.388	0.191	0.328	0.519	-0.131	1.233	0.607	0.503	1.110	+0.123
11	0.399					1.205				
Av.	0.383	0.156	0.303	0.459	-0.074	1.157	0.750	0.468	1.138	+0.012

Series B

1	1.118	0.217	0.640	0.857	+0.261	1.633	0.919	0.457	1.375	+0.258
2	1.090	0.208	1.076	1.285	-0.195	1.608	0.912	0.769	1.681	-0.073
3	1.087	0.186	1.106	1.292	-0.205	1.655	0.891	0.745	1.637	+0.018
4	1.093	0.255	0.999	1.254	-0.161	1.617	0.833	0.603	1.436	+0.181
5	1.079	0.235	0.957	1.192	-0.113	1.659	0.844	0.687	1.531	+0.128
6	1.101	0.210	1.107	1.317	-0.216	1.652	0.895	0.769	1.664	-0.012
Av.	1.095	0.219	0.981	1.200	-0.105	1.637	0.882	0.672	1.554	+0.083

Series C

1	0.193	0.114	0.218	0.332	-0.139	0.913	0.625	0.376	1.001	-0.088
2	0.196	0.114	0.192	0.306	-0.110	0.925	0.572	0.421	0.993	-0.068
3	0.192	0.103	0.162	0.265	-0.073	0.967	0.609	0.381	0.990	-0.023
4	0.187	0.100	0.197	0.298	-0.111	0.964	0.644	0.415	1.059	-0.095
5	0.188	0.138	0.197	0.335	-0.147	0.924	0.567	0.415	0.982	-0.058
Av.	0.191	0.114	0.193	0.307	-0.116	0.939	0.603	0.402	1.005	-0.066

TABLE III (CONTINUED)

Subject 4 - Weight 83 to 84 Kilograms

Series A

Period	Calcium					Phosphorus				
	Intake	Output			Balance	Intake	Output			Balance
		Urine	Feces	Total			Urine	Feces	Total	
1	0.394					1.140				
2	0.364					1.081				
3	0.367	0.063	0.293	0.356	+0.011	1.117	0.600	0.411	1.011	+0.106
4	0.385	0.098	0.311	0.409	-0.024	1.112	0.501	0.416	0.917	+0.195
5	0.383	0.087	0.312	0.399	-0.016	1.156	0.711	0.398	1.109	+0.047
6	0.375	0.094	0.353	0.447	-0.072	1.125	0.629	0.498	1.127	-0.002
7	0.385	0.122	0.395	0.517	-0.132	1.198	0.610	0.534	1.144	+0.054
8	0.395	0.077	0.296	0.373	+0.022	1.160	0.644	0.410	1.054	+0.106
9	0.380	0.090	0.306	0.396	-0.016	1.195	0.699	0.412	1.111	+0.084
10	0.388	0.102	0.441	0.543	-0.155	1.233	0.644	0.612	1.256	-0.023
11	0.399	0.101	0.379	0.480	-0.081	1.205	0.727	0.468	1.195	+0.010
Av.	0.383	0.093	0.343	0.436	-0.051	1.157	0.641	0.462	1.103	+0.064

Series B

1	1.081	0.135	0.735	0.870	+0.211	1.511	0.765	0.582	1.347	+0.164
2	1.053	0.103	0.885	0.988	+0.068	1.486	0.902	0.642	1.544	-0.058
3	1.051	0.116	0.993	1.109	-0.058	1.533	0.931	0.692	1.623	-0.090
4	1.056	0.125	0.949	1.074	-0.018	1.495	0.766	0.641	1.407	+0.088
5	1.042	0.118	1.013	1.131	-0.089	1.537	0.649	0.745	1.394	+0.143
6	1.065	0.067	1.077	1.144	-0.079	1.530	0.822	0.797	1.619	-0.089
Av.	1.058	0.111	0.942	1.053	+0.005	1.515	0.806	0.683	1.489	+0.026

Series C

1	0.194	0.037	0.456	0.493	-0.299	0.913	0.531	0.622	1.153	-0.240
2	0.196	0.042	0.319	0.361	-0.165	0.925	0.542	0.516	1.058	-0.133
3	0.191	0.046	0.325	0.371	-0.180	0.967	0.425	0.492	0.917	+0.050
4	0.186	0.043	0.325	0.368	-0.182	0.964	0.510	0.492	1.002	-0.038
5	0.188	0.053	0.331	0.384	-0.196	0.924	0.535	0.525	1.060	-0.136
Av.	0.191	0.044	0.351	0.395	-0.204	0.939	0.509	0.529	1.038	-0.100

TABLE IV
EFFECT OF ABRUPT CHANGES IN CALCIUM INTAKE
ON CALCIUM BALANCES

Subject	Series	Periods	Number of Days	Calcium in Grams			
				Average Daily Intake	Total Storage	Total Loss	Average Daily Balance
1	A	1-2 } 5-11)	27	0.351		1.536	-0.057
	B	1-2	6	1.067	0.506		+0.084
		3-6	12	1.057		0.540	-0.045
	C	1	3	0.194		0.458	-0.153
		2-5	12	0.191		0.961	-0.080
	Net		60			2.987	-0.050
2	A	1-4 } 6-11)	30	0.351		0.724	-0.024
	B	1	3	1.081	0.437		+0.146
		2-6	15	1.057		0.150	-0.010
	C	1	3	0.194		0.504	-0.168
		2-4	9	0.192		0.686	-0.076
	Net		60			1.627	-0.027
3	A	1-4 } 6-10)	27	0.381		2.095	-0.078
	B	1	3	1.118	0.782		+0.261
		2-6	15	1.093		2.668	-0.178
	C	1-5	15	0.191		1.739	-0.116
	Net		60			5.720	-0.095
4	A	3-11	27	0.385		1.389	-0.051
	B	1-2	6	1.067	0.837		+0.139
		3-6	12	1.057		0.731	-0.061
	C	1	3	0.194		0.898	-0.299
		2-5	12	0.191		2.172	-0.181
	Net		60			4.353	-0.073

TABLE V

AVERAGE DAILY PHOSPHORUS BALANCES ON VARYING ADEQUATE
LEVELS OF INTAKE PARALLELING MARKED CHANGES
IN INTAKE OF CALCIUM

Subject	Series	Periods	Number of Days	Av. Daily Intake of Calcium	Phosphorus	
					Av. Daily Intake	Av. Daily Balance
1	A	1-2 } 5-11 }	27	0.351	1.129	+0.064
	B	1-2	6	1.067	1.499	+0.071
		3-6	12	1.057	1.522	+0.033
	C	1 2-5	3 12	0.194 0.191	0.913 0.945	-0.135 -0.081
	Net		60			+0.020
2	A	1-4 } 6-11 }	30	0.351	1.124	+0.012
	B	1	3	1.081	1.511	+0.107
		2-6	15	1.057	1.516	+0.056
	C	1 2-4	3 9	0.194 0.192	0.913 0.939	-0.164 -0.071
	Net		60			+0.007
3	A	1-4 } 6-10 }	27	0.381	1.156	+0.011
	B	1	3	1.118	1.633	+0.256
		2-6	15	1.093	1.638	+0.026
	C	1-5	15	0.191	0.939	-0.066
	Net		60			+0.024
4	A	3-11	27	0.385	1.167	+0.064
	B	1-2	6	1.067	1.499	+0.111
		3-5	12	1.057	1.522	+0.013
	C	1 2-5	3 12	0.194 0.191	0.913 0.945	-0.240 -0.064
	Net		60			+0.018

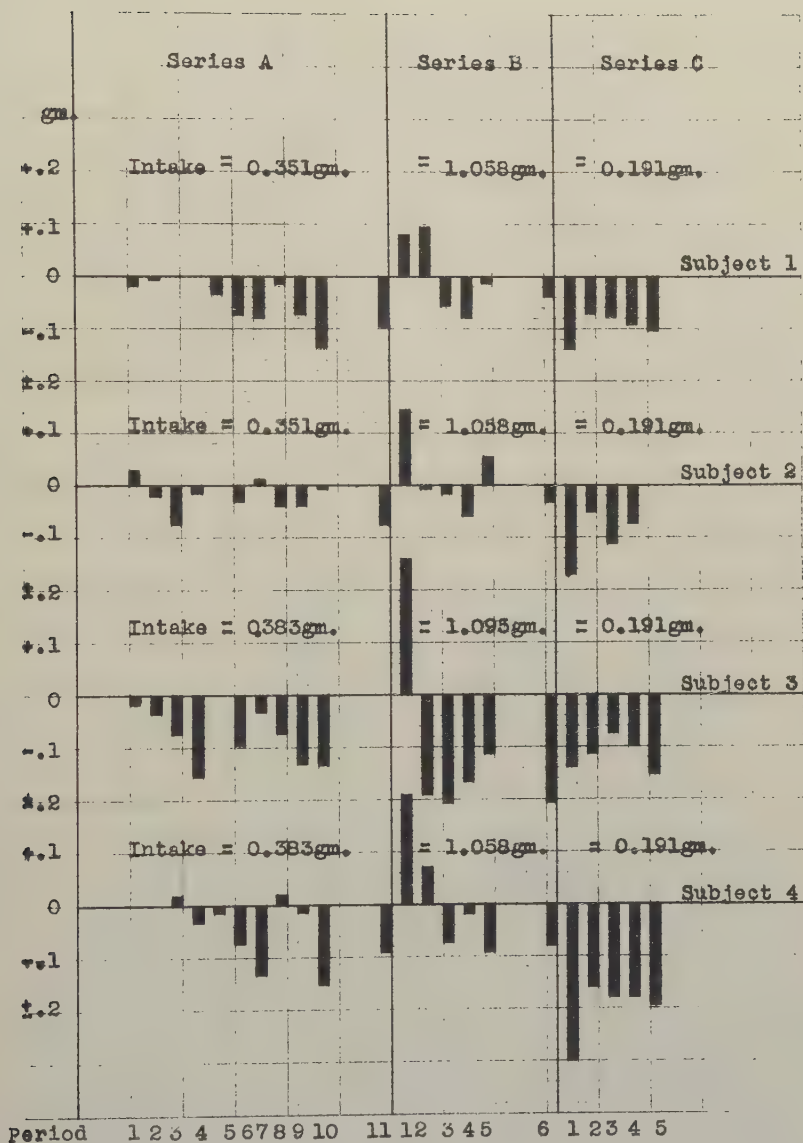


Fig.1.--Calcium balances of normal adults following abrupt and marked changes in level of intake.

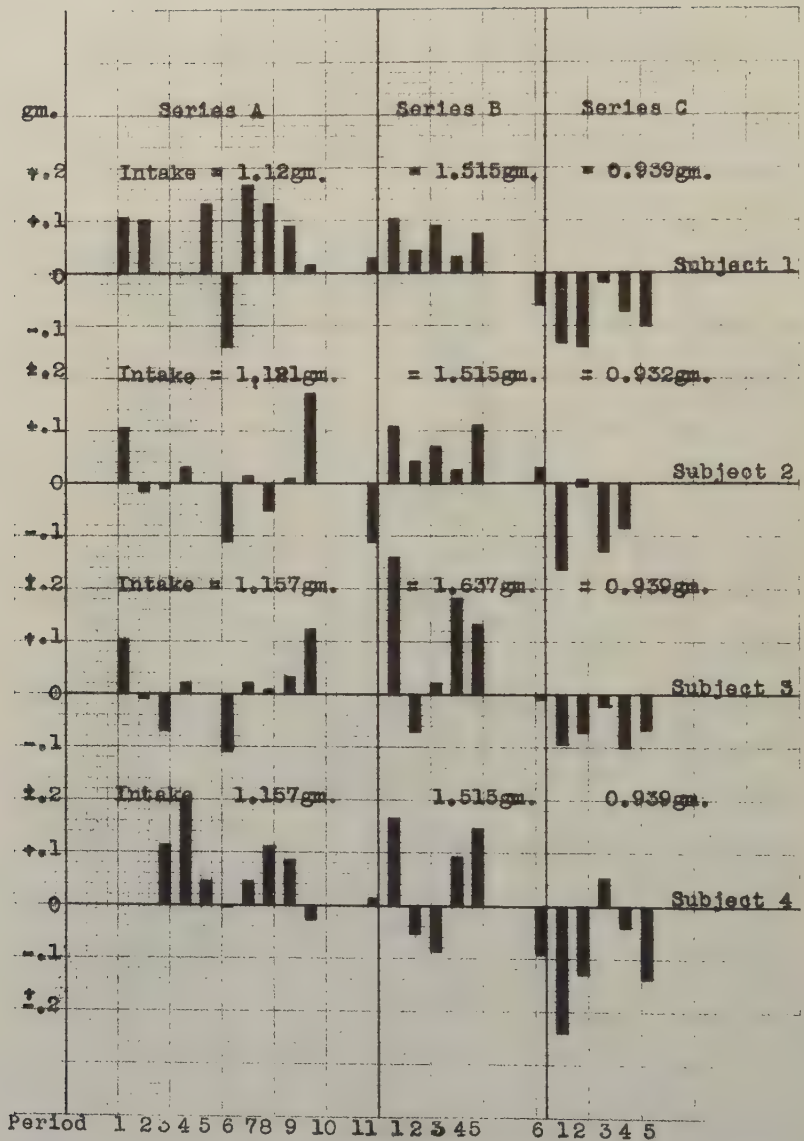


Fig.2.--Phosphorus balances of normal adults on varying adequate levels of intake paralleling marked changes in intake of calcium.

0.080 grams, 0.076 grams, and 0.181 grams daily for these same subjects. In the case of Subject 3 the loss sustained during the first period fell within the range of the losses during the later periods of the series and the average daily loss for the 15 days was 0.095 grams.

These results seem to raise a definite question as to the validity of the general assumption that the total calcium excretion on a level of intake which results in a slight negative balance represents the requirement of the mineral for maintenance of equilibrium. Sherman (23) observed that at very low levels of intake calcium excretion was suppressed, but assumed that as negative balances became small this evident conservation of the mineral would be insignificant. Paget, Langeron, and Cordonnier (19) have more recently noted that with the experimental conditions of their studies, calcium excretion varied less than the changes in intake on both sides of a point of physiological equilibrium. As far as can be ascertained there was in the present study no dietary or environmental difference that could be considered responsible for the fact that negative balances were practically the same on intakes of approximately 0.4 and 1.1 grams daily. It seems rather that, with these subjects, the body stores of the mineral were so bountiful that, over a wide range of intake, there was a tendency to throw it off. However, it is to be noted that when the amounts excreted on intakes of approximately 0.4 grams of calcium daily in Series A, and 0.2 grams in Series C are compared, that with all of the subjects, though in varying degrees, the total calcium excretion varied less than the difference in the intake. This is shown even more specifically in the case of Subject 2 when the results of these two series are assembled with the results of the preliminary 24-day study previously referred to and given in Table X. As noted elsewhere, an effort had been made to stabilize the calcium stores of this subject by the use of a low calcium intake over an appreciable period of time, and when the results of the three series of balances, made at levels of intake which caused definite calcium losses, are compared as in Table VI, it is seen that where the calcium intake was raised from 0.192 grams to 0.288 grams daily, the increased intake of 0.096 grams decreased the daily negative balance by only 0.033 grams and again, an increase in intake of 0.063 grams, when the level of intake was raised from 0.288 grams to 0.351 grams decreased the negativity of the daily balance by only 0.019 grams. It is therefore quite evident that in the case of this subject an increase in calcium intake at low levels decreased the negativity of the resulting balance approximately one-third as much as the intake was increased.

TABLE VI
THE EFFECT OF INCREASES IN THE CALCIUM INTAKE AT
LOW LEVELS ON THE CALCIUM BALANCES

Subject	Series	Daily Averages			
		Intake	Balance	Increase in Intake	Decrease in Negative Balance
		gms.	gms.	gms.	gms.
2	C	0.192	-0.076		
	P	0.288	-0.043	0.096	0.033
	A	0.351	-0.024	0.063	0.019

The Effect of Previous Dietary History
on Calcium Balances

In considering the response of the four subjects to the different levels of calcium intake, the previous dietary histories and their probable effect upon the calcium stores of the subjects should be borne in mind. Recognizing that the work of Bauer, Aub, and Albright (2) had demonstrated that calcium may be stored in, or withdrawn from bone trabeculae with considerable ease, Subject 2 had, as has been previously noted, for six months prior to the beginning of the present experiment, endeavored, by means of a low calcium intake, to minimize any stores of calcium which might be considered to be loosely held, and thus make herself sensitive to changes in level of intake. It was hoped that by so doing she might, in a way, serve as a control for other subjects. It has also been already pointed out that in marked contrast to Subject 2, the other three subjects used in the study had all been taking large amounts of calcium for a long time previous to the beginning of the study. It therefore seems reasonable to suppose that the latter all had very high and possibly maximum stores of body calcium.

In Series A calcium equilibrium was expected, for the amount of calcium in the diets had been based on the total excretion of Subject 2 in a preliminary study, and it is quite generally assumed that the total excretion of a mineral on an intake that produces small negative balance represents the requirement for maintenance. As the calcium stores of the other subjects were believed to be high, it was expected that they would have a lower requirement of the mineral, so that equilibrium by all of the subjects was fully anticipated. The results, however, present an en-

tirely different picture. While the negative balances of Subject 2 were definitely less than in the previous study on a lower intake, they cannot, in consideration of the length of the series, be interpreted as representing equilibrium. Moreover, the other three subjects, with a marked similarity to each other, all showed by appreciably higher negative balances than Subject 2, a greater tendency to give up the mineral, thus suggesting a higher requirement for maintenance of equilibrium than the balances of Subject 2 indicate.

A similar difference between the subjects is to be seen in their response to the increased intake of over a gram of calcium a day in Series B. At this level of intake, Subject 2 in one 3-day period stored an amount of calcium equivalent to more than 18 days' loss in Series A, and then remained in practical equilibrium for the remainder of the series. Subjects 1, 3, and 4, however, showed no tendency to compensate for the losses that they had sustained during the previous six weeks, but after temporary retention for from three to six days reverted to definite negative balances. Unfortunately collections in Series B were not continued long enough for definite conclusions to be drawn as to the ultimate reaction of these subjects to this level of intake. Two possibilities may be considered: one, that the calcium of this initial retention was loosely held and that in the succeeding periods there was a tendency to give up this mineral gradually, and very shortly attain equilibrium at this level; the other, that even this comparatively high level of intake was not adequate for the maintenance of stores that had been laid down by these subjects just previously, during months of active life with an abundance of sunshine and an even higher level of calcium intake, and that consequently an appreciable length of time would have been required for them to equilibrate their body stores to the new conditions. The latter idea may seem improbable, but the fact that after a two weeks interval on approximately the same level of intake, and a 3-day preliminary period on the same weighed diet, all of the subjects still showed negative balances at least suggests it as a possibility. The large losses of calcium by Subject 3 in this series are difficult to explain, although several similar cases are reported in the literature. Hunscher, Donelson, Erickson, and Macy (14) have recently reported large negative balances on two normal subjects with intakes of calcium in excess of one gram daily; Bogert and McKittrick (6) found little difference in the calcium balances of two of their subjects when the daily intake of the mineral was increased from 0.266 grams to 1.035 grams daily by the addition of calcium lactate; Donelson, Nims, Hunscher, and Macy (10) have reported that more than forty weeks after cessation

of lactation two non-pregnant mothers excreted daily 0.76 and 0.91 grams more than their intakes, which had amounted to 1.75 and 1.68 grams respectively. In discussing large losses of calcium by a pregnant woman, Macy and her co-workers (17) say that, in their opinion, the effect of mental state on physiological processes is indicated. Whether or not emotional condition can be ascribed as a cause, it is a fact that during this series of balances Subject 3 was greatly worried, and in an extremely high-strung, nervous condition.

The results obtained in Series C are strikingly alike for all subjects. As previously noted, on an intake of calcium which averaged 0.191 grams daily, after one period of comparatively high excretion, the averages of the daily balances of Subjects 1, 2, 3, and 4 were -0.080, -0.074, -0.110, and -0.181 grams respectively, the total excretion per kilo of body weight being 4.4 milligrams for Subjects 1, 3, and 4, and 4.1 milligrams per for Subject 2. The last figure is in exact agreement with the average obtained by Bauer, Albright, and Aub (1) from forty-six 3-day balances on normal individuals with an average intake of 0.11 grams of calcium daily.

From the consideration of these results it seems apparent that the differences in the calcium utilization of the four subjects may, with considerable assurance, be ascribed to the difference in the state of their calcium stores. With Subjects 1, 3, and 4 considerable amounts of the mineral were apparently loosely held and easily given up, while Subject 2 at all times much more nearly approached equilibrium, indicating presumably greater stability of the stored mineral. It is to be noted, however, that when the calcium intake was dropped to a very low level in Series C the output of Subjects 1, 3, and 4 decreased to nearly the same rate of excretion as Subject 2. This suggests that this loosely-held calcium, which is probably stored largely in the vascular bone trabeculae, is conserved under such conditions, and when the amount of calcium ingested remains low for any appreciable length of time, or presumably, if for any reason absorption is poor or excretion high, it serves as a very definite protection against depletion of more stable stores such as bones and teeth. Because of the nature of their previous diets, Subjects 1, 3, and 4 had bountiful stores of calcium and a very high intake of mineral would have been required to preserve them without loss. It therefore seems that with adults positive calcium balances at a low level of intake, instead of indicating a low requirement, imply rather a greater need for the mineral, or at least a greater ability to use or store calcium supplied in excess of immediate needs

for vital metabolic processes. Wang (26) and Daniels (9) have found, similarly, that the amount of calcium retained by a child on an adequate or luxus consumption of milk depends on the physiological condition of the child, and when the previous diet has been below the child's requirements more of the mineral is retained than when the diet has previously been adequate.

The fact that the human adult can, under some conditions, store huge amounts of calcium is clearly shown by the work of Bauer, Marble, and Claflin (3). One of their subjects, after a preliminary period of ten days on an intake of 1.33 grams of calcium per day, stored over 0.2 grams of the mineral daily for 39 days at this level of intake, the other, with a preliminary period of similar length, on a daily intake of 1.49 grams stored more than 0.5 grams of calcium daily for 69 days. These balances were not significantly affected by the administration of irradiated ergosterol for part of the time. That such high rate of storage would continue indefinitely is inconceivable, and not borne out by historical evidence.

It is believed that these results suggest an explanation for the confusion that exists because of the widely varying amounts of calcium that have been proposed as the requirement for maintenance of equilibrium in adults. Because most calcium balance studies have been made for the purpose of studying the availability of calcium from various sources, or the factors affecting its utilization, the allowance of the mineral has generally been kept somewhat low as this would presumably favor the manifestation of any differences. Sherman (22) assumed that the total excretion by a subject, on an intake that causes a slight negative balance represents the minimum requirement for equilibrium, and in his analysis of the data then available used only studies which showed practical equilibrium or negative balances, by far the most being of the latter order. In many of the balance periods used the negative balances amounted to 0.1 grams per day and in several were as high as 0.2 grams. As it has already been pointed out, the results of the present study confirm the observations of Paget and his co-workers in that at low levels of calcium intake negative balances varied less than the intakes from which they resulted, so that it does not seem that one could ever assume that the total calcium excretion in such cases truly represents the requirement of the mineral for equilibrium. Paget and his associates (19) by using different levels of calcium allowance with the same subjects came to the conclusion that 0.85 grams of calcium daily is the so-called "ration of equilibrium." Manoussakis (18), who in an intensive 69-day study on a single individual also determined the excretion at varying levels of intake, concluded that

one gram of calcium a day was needed to maintain this subject in equilibrium. It is clearly evident that at high levels of intake calcium is not usually used economically and it is a question whether or not, in the adult, maximum stores of loosely-held calcium which require excessively high intakes for their maintenance are a physiological asset.

The danger of attempting to generalize from fragmentary evidence is keenly appreciated, nevertheless, from the results obtained in the present study the following theory is tentatively proposed: that the calcium requirement for maintenance of equilibrium by a human adult is dependent, not only upon the amount required for maintenance of vital metabolic processes and the availability of the calcium ingested, but also upon the state of the body stores of the individual, as determined by the previous diet over long periods of time, and the higher the calcium content of this previous diet, the higher also will be the requirement of the mineral for maintenance of equilibrium.

Physiological Adjustment to Changed Levels of Calcium Intake

From the foregoing discussion of the results of the study it is quite evident that the human body does not immediately adjust itself to changes in the amount of ingested calcium, a fact generally recognized by recent workers in the field, so, in an effort to study the nature and duration of this adjustment, the effects on the calcium balances of the two abrupt changes made during the study were critically inspected. By reference to Table IV and Figure 1 it is seen that immediately following the change from the moderate intake of between 0.35 and 0.4 grams daily in Series A to the liberal allowance of approximately 1.1 grams in Series B, there was with all subjects at first definite retention of the mineral which was not characteristic of later periods. In the case of Subject 2 a positive balance in the first 3-day period largely compensated for the loss of the mineral sustained in Series A, but after this three days of storage this subject remained in equilibrium during the remainder of the series. Subject 3 also stored calcium for one period of Series B and throughout the remainder of the series sustained continued high losses. The other two subjects stored calcium during the first two periods of Series B, but then reverted to negative balances. There was, unquestionably, a conspicuous difference in the trend of the first one or two balances of Series B and those which followed. Similarly, after the sudden change from the high calcium allowance of Series B to the low intake of less than 0.2 grams daily in Series C there was, with Subjects 1, 2, and 4, a comparatively high rate of loss

for one period, then, in an apparent effort at conservation, the excretion was diminished and maintained at a fairly constant level during the remainder of the series. In the case of Subject 3 the differences between the balances of the first and later periods were insignificant. It is a matter of interest that in all cases urinary excretion of calcium at once changed to the new levels, indicating, presumably, that absorption and renal excretion quickly adapt themselves to new conditions and that delayed adjustment is due to an inability of the intestinal excretory mechanism to make rapid changes in its rate of functioning. In their practical application the results indicate that calcium balance studies should be preceded by preliminary period of from three to six days on the experimental diet, but that a longer period is of no advantage, and an unnecessary expense.

Normal Variations in the Magnitude of Calcium Balances on a Constant Intake

It seems obvious that the evaluation of differences in calcium balance experiments, as well as the length of period which will give a reliable sample of calcium utilization will depend upon the normal variation that may be expected when an individual is on a constant diet. The results of the present study were therefore critically inspected in an attempt to arrive at some measure of this variation. Comparison of the average deviation of the balances of 3-day periods from the mean balances of the series in which they occurred showed them to be of the same order of magnitude as those of similar studies by other workers, where the results of three or more consecutive balance periods on a constant diet have been reported. Consequently the data from consecutive 3-day balance periods in Series A were combined so as to obtain balances from all possible 6-day and 9-day periods, and the average deviations of these balances from the mean of the 30-day balance of the entire series computed. The result of this treatment is shown in Table VII. It is seen that, as is to be expected, the deviations decrease as the experimental period is lengthened, and in the 9-day periods are so small as to be apparently insignificant. For this reason it would seem that a 9-day balance period should give a fairly reliable sample of calcium utilization by normal adults living a regular routine life, with a constant intake of the mineral.

Relation of Urinary Calcium to Total Calcium Excretion

Inspection of the data on calcium excretion in the present

TABLE VII

COMPARISON OF THE AVERAGE DEVIATIONS OF THE AVERAGE DAILY CALCIUM BALANCES OF PERIODS OF VARYING LENGTH FROM THE MEAN OF 27 TO 30 DAYS BALANCES ON A CONSTANT DIET

Subject	Average Deviations		
	3-day Period	6-day Period	9-day Period
	gms.	gms.	gms.
1.....	0.035	0.023	0.006
2.....	0.027	0.015	0.004
3.....	0.041	0.033	0.019
4.....	0.050	0.042	0.016

study reveals an interesting physiological consistency in the case of each subject, as well as a constant variation between the different subjects in the proportion of the total calcium excretion that is eliminated in the urine, as shown in Table VIII. Excluding the adjustment periods, the urinary calcium for the respective subjects amounted to 46 per cent, 29 per cent, 34 per cent, and 21 per cent of the total calcium excreted in Series A; 25 per cent, 15 per cent, 17 per cent, and 10 per cent in Series B; and 48 per cent, 31 per cent, 37 per cent, and 12 per cent in Series C. Except in the case of Subject 4 in Series C, where a slight but definite difficulty in fat digestion had been recognized but disregarded, these percentages in the different series bear the same relation to each other and when plotted form approximately parallel and nearly straight lines, as shown in Figure 3. A similar relationship is to be found between the urinary calcium and the total excretion in the control periods of the two subjects of Bauer, Marble, and Claflin (3), who were studied at two levels of intake.

Phosphorus Balances

While calcium and phosphorus are definitely associated in skeletal changes, other maintenance functions are greatly different from each other, and there is little relationship to be noted under the experimental conditions of the present study. As has been previously indicated, the phosphorus allowance in all series was planned to meet the maintenance requirements of the mineral,

TABLE VIII

RELATION OF URINARY CALCIUM TO TOTAL CALCIUM EXCRETION

Series	Subject	Average Daily Total Calcium Excretion	Urinary Calcium		Ratio of Percentages
			Daily Average	Per cent of Total	
		gms.	gms.		
A	1	0.410	0.190	46.4	1.00
	2	0.375	0.108	28.8	0.62
	3	0.459	0.154	33.5	0.72
	4	0.436	0.093	21.3	0.46
B	1	1.099	0.269	24.5	1.00
	2	1.063	0.160	15.0	0.61
	3	1.268	0.219	17.2	0.71
	4	1.115	0.107	9.6	0.39
C	1	0.271	0.131	48.4	1.00
	2	0.268	0.084	31.2	0.64
	3	0.307	0.114	37.1	0.76
	4	0.371	0.046	12.4	0.26
Bauer and others (4)	R. L.	0.190	0.130	65.0	1.00
	M. A. R.	0.180	0.080	44.0	0.68
	R. L.	0.930	0.290	31.0	1.00
	M. A. R.	1.140	0.250	22.0	0.70

but for practical reasons was not maintained at the same level in all series but varied somewhat in the same direction as the changes in the calcium intake. By reference to the complete data given in Table III, to the summary of the phosphorus balances shown in Table V, and to Figure 2 where the balances are presented graphically, it is seen that in Series A, on an average intake of 1.1 grams daily, Subjects 1, 2, 3, and 4 stored respectively 0.064 grams, 0.012 grams, 0.011 grams, and 0.064 grams daily for the series. In Series B, with the intake approximating 1.5 grams daily, in the transition periods the daily retention by the subjects in the same order amounted to 0.071 grams, 0.107 grams, 0.256 grams, and 0.111 grams, but in the later periods of the series it dropped to give average daily positive balances of 0.033 grams, 0.056 grams, 0.026 grams, and 0.013 grams for these same subjects. With the daily intake lowered to 0.9 grams in Series C, average daily losses of

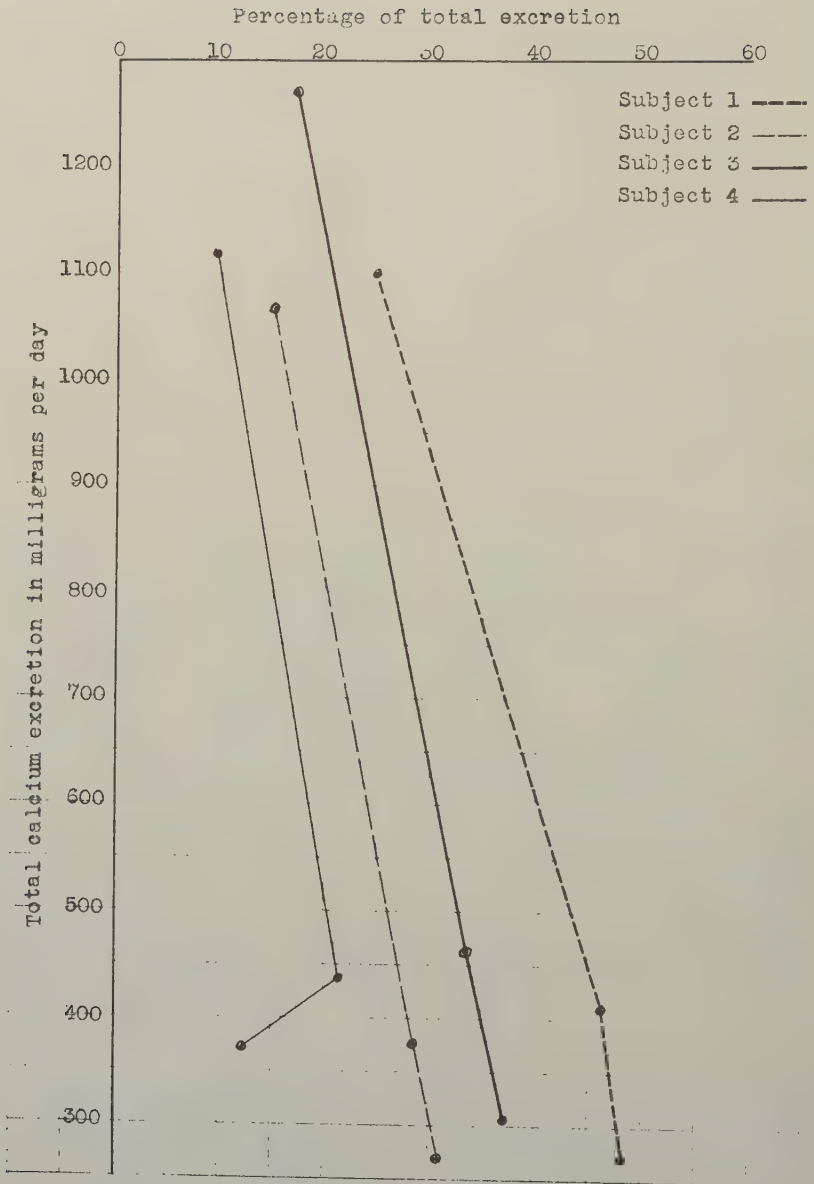


Fig.3.--Relation of urinary calcium to total calcium excretion.

0.135 grams, 0.164 grams, and 0.240 grams were shown by Subjects 1, 2, and 4 in the first period, but in the later periods these losses were reduced to 0.081 grams, 0.071 grams, and 0.064 grams daily. As with calcium, Subject 3 showed no difference in the phosphorus loss of the first period and that sustained subsequently, the loss for the entire series averaging 0.066 grams daily. The net result of the sixty days' collections was a slight daily positive balance for all subjects.

It is to be noted that in Series A and in the periods of Series B when there was slight calcium losses, phosphorus was retained, indicating little relationship. However, in the adjustment periods of Series B when calcium was retained there was increased storage of phosphorus, and in Series C the higher losses of calcium by three of the subjects in the first period was paralleled by higher loss of phosphorus. These facts suggest that, in even temporary retention and loss of calcium, stores of calcium phosphate, presumably in bone, are definitely affected, and under ordinary conditions of life are constantly undergoing change.

Relation of Vitamin D to Calcium Balances

The possibility of any effect from the lack of Vitamin D in the present study should also be considered. It is to be recognized that the study took place in the city in the winter months, and the diets, in spite of a liberal allowance of butter in all series, probably contained an insignificant amount of this vitamin. It does not seem justifiable, however, in the present state of our knowledge, to assume that this had any bearing on the results, for it has been shown rather conclusively by short experiments in this laboratory (8, 15) as well as by the extended investigations of Hart, Tourtellotte, and Heyl (13), Bauer, Marble, and Claflin (3), and Hunscher, Donelson, Erickson, and Macy (14) that Vitamin D added to the diet of normal individuals has no effect on the magnitude of calcium balances. It would therefore seem that either Vitamin D requirements for maintenance are very low, or that when Vitamin D stored in the body is adequate for immediate needs, the effect of an excess is not apparent. The latter idea is purely theoretical, however, for while a species difference in the ability to store Vitamin D is recognized, nothing at all is known of it in regard to human beings.

SUMMARY

The calcium metabolism of four normal, healthy college women was studied over a period of three months by means of calcium balances. Twenty 3-day collections of food and excreta were

made for each at three levels of calcium intake, which approximated 0.2 grams, 0.4 grams, and 1.1 grams daily, with the other food essentials maintained at presumably adequate, and fairly constant levels at all times. Three of the subjects had continued to take their usual diets, which included liberal quantities of milk, until the beginning of the study; the other, although accustomed to a high calcium intake, had, for six months prior to the beginning of the experiment been on a restricted calcium allowance.

The purpose of the investigation was: (1) to study the calcium utilization of the same subjects on varying levels of calcium intake; (2) to determine, if possible, any effects of previous dietary history on calcium utilization, particularly as it might affect the calcium requirement for maintenance of equilibrium; (3) to observe the nature and duration of physiological adjustment to changed levels of calcium intake; and (4) to find the magnitude of the variation that might normally be expected in periods of varying length on a constant diet and thus find the length of period which would give a representative sampling of calcium utilization on a constant intake under ordinary living conditions.

It was found, unexpectedly, that negative calcium balances were obtained at all three levels of intake. With a daily ingestion of 0.2 grams of calcium, all of the subjects excreted slightly more than four milligrams per kilo of body weight per day; with the intake raised to 0.4 grams of the mineral daily, the negative balances, while less, were not decreased proportionately to the increase in the intake; on the liberal allowance of 1.1 grams of calcium daily, the subject who had previously been on a low calcium intake attained practical equilibrium, while the other subjects continued to show definite losses.

Because the subject who had just previously been on a low calcium intake approached calcium equilibrium on both moderate and high levels of intake while the other subjects all continued to show definite calcium losses it is proposed, as a tentative theory, that the calcium requirement for maintenance of equilibrium by a human adult is dependent not only upon the amount of the mineral needed for the maintenance of vital metabolic processes, but also upon the state of the body stores of the mineral as determined by the previous diet over long periods of time, and that the higher the calcium content of this previous diet, the higher also will be the requirement of the mineral for maintenance.

Immediately following abrupt changes in the level of calcium intake there was delayed adjustment in intestinal excretion of the mineral so that the balances of the first one or two 3-day periods after the change were not truly representative of the utilization of the mineral, as shown by the balance of subsequent pe-

riods on the same intake. The results show the necessity of at least a 3-day preliminary period on the experimental diet before beginning collections, for a calcium balance study, and indicate that, while a slightly longer period may be desirable, a preliminary period longer than six days is of no advantage.

The average deviations of the daily balances of 3-day balance periods from the mean of 27 to 30 days' balances on the same intake ranged from 0.03 grams to 0.05 grams for the different subjects; for 6-day periods the range was from 0.015 to 0.04 grams, and in 9-day periods it was for all subjects less than 0.02 grams. This suggests that a 9-day balance period gives a representative sampling of calcium utilization for a much longer period under similar conditions.

Inspection of the data on excretion disclosed an individual consistency and a consistent variation between subjects in the proportion of the total calcium excretion that was eliminated in the urine.

APPENDIX

Preliminary Study

Three months previous to the study just described, Subject 2 after three months on a low calcium diet estimated to contain less than 0.25 grams of calcium daily undertook a calcium balance study on a constant diet as given in Table IX. The food

TABLE IX
DIET USED DAILY IN THE PRELIMINARY STUDY
(SERIES P)

Food	Weight gms.
Beef	150
Whole wheat bread	140
Shredded wheat	35
Potato	180
Lettuce	100
Orange juice	77
Banana	100
Apple	100
Butter	70
Milk	90

value of this diet as calculated from the tables of Sherman (22) and Rose (20) is: calories, 1,720; protein, 60.7 grams; calcium, 0.32 grams; phosphorus, 0.97 grams; and iron, 0.012 grams. Sugar was used as desired to supplement the calories. Following a six-day preliminary period on the weighed diet, collections of food composites and excreta were made for eight consecutive 3-day periods designated Series II. The techniques used were similar to those previously described except that, instead of drying the foods and feces, acid digests were made. Food composites representing one-fifth of the day's intake were digested over a low flame with 30 cc. of concentrated sulphuric acid, and the composites for three days, after being passed through a very fine strainer, were combined and made up to 1,000 cc. The combined feces for each 3-day period were boiled with ten per cent sulphuric acid until emulsified, then strained and made up to volume. In the case of both

digest any material that remained on the strainer at first was boiled with concentrated acid until so disintegrated that it would pass through. Measured portions of the digests were evaporated and ashed for the chemical analyses. The results of the analyses are shown in Table X.

TABLE X
SUMMARY OF CALCIUM AND PHOSPHORUS DATA FROM
PRELIMINARY STUDY--DAILY AVERAGES IN GRAMS

Subject 2--Weight 64 Kilograms										
Period	Calcium					Phosphorus				
	In-take	Output			Balance	In-take	Output			Balance
		Urine	Feces	Total			Urine	Feces	Total	
P-1	0.302	0.067	0.198	0.265	+0.037	1.000	0.588	0.307	0.895	+0.105
2	0.278	0.078	0.284	0.362	-0.083	0.895	0.559	0.434	0.993	-0.098
3	0.293	0.070	0.240	0.311	-0.018	0.883	0.670	0.361	0.031	-0.148
4	0.274	0.088	0.262	0.350	-0.076	0.899	0.427	0.365	0.792	+0.107
5	0.300	0.099	0.243	0.342	-0.042	1.008	0.696	0.354	1.050	-0.042
6	0.274	0.107	0.230	0.337	-0.063	0.827	0.540	0.371	0.911	-0.084
7	0.287	0.102	0.266	0.368	-0.181	0.840	0.563	0.327	0.890	-0.050
8	0.298	0.083	0.235	0.318	-0.020	0.873	0.529	0.332	0.862	+0.011
Av.	0.288	0.087	0.244	0.331	-0.043	0.903	0.571	0.356	0.927	-0.024

It is seen that with an average daily intake of 0.288 grams of calcium for the twenty-four days of the study, the urinary excretion of the mineral averaged 0.087 grams and that excreted by the fecal path 0.244 grams daily, resulting in an average daily negative balance of 0.043 grams. These results have been previously referred to at different times, and the data used in the statistical work, so need no further discussion.

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